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α_2-Macroglobulin as a β-Amyloid Peptide-Binding Plasma Protein

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Abstract: The β-amyloid peptide (Aβ) is a normal proteolytic processing product of the amyloid precursor protein, which is constitutively expressed by many, if not most, cells. For reasons that are still unclear, Aβ is deposited in an aggregated fibrillar form in both diffuse and senile plaques in the brains of patients with Alzheimer's disease (AD). The factor(s) responsible for the clearance of soluble Aβ from biological fluids or tissues are poorly understood. We now report that human α_2-macroglobulin (α_2M), a major circulating endoproteinase inhibitor, which has recently been shown to be present in senile plaques in AD, binds ^{125}I-A$\beta_{(1-42)}$ with high affinity (apparent dissociation constant of 3.8 $\times 10^{-10}$ *M*). Approximately 1 mol of Aβ is bound per mole of α_2M. Both native and methylamine-activated α_2M bind ^{125}I-A$\beta_{(1-42)}$. The binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M is enhanced by micromolar concentrations of Zn^{2+} (but not Ca^{2+}) and is inhibited by noniodinated A$\beta_{(1-42)}$ and A$\beta_{(1-40)}$ but not by the reverse peptide A$\beta_{(40-1)}$ or the cytokines interleukin 1β or interleukin 2. α_1-Antichymotrypsin, another plaque-associated protein, inhibits both the binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M as well as the degradation of ^{125}I-A$\beta_{(1-42)}$ by proteinase-activated α_2M. Moreover, the binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M protects the peptide from proteolysis by exogenous trypsin. These data suggest that α_2M may function as a carrier protein for Aβ and could serve to either facilitate or impede clearance of Aβ from tissues such as the brain.
Key Words: β-Amyloid peptide—Amyloid precursor protein—Alzheimer's disease—Senile plaques—α_2-Macroglobulin—Brain.
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α_2-Macroglobulin (α_2M), present in high concentrations in human plasma, is a 720-kDa glycoprotein consisting of four identical (180-kDa) subunits (Sottrup-Jensen, 1989). α_2M has been shown to subserve a unique trapping mechanism both in vitro and in vivo, irreversibly capturing a variety of proteinases, resulting in "pan-proteinase" inhibitory activity, especially for large protein substrates (Ganrot, 1966; Barrett and Starkey, 1973). By contrast, proteinase-activated α_2M is able to efficiently degrade smaller substrates (Haverback et al., 1962). In addition to binding and capturing proteinases, α_2M also binds a number of polypeptide growth factors and cytokines (James, 1990; Borth, 1992). Although the exact physiological significance of the binding of these peptides or cytokines to α_2M is uncertain, it has been shown that such binding protects smaller peptide substrates from proteolytic degradation (Legrés et al., 1995). Furthermore, the clearance and catabolism of these peptides or cytokines may be enhanced after the Ca^{2+}-dependent binding and endocytosis of α_2M via its well-described receptor, the α_2M receptor/low-density lipoprotein receptor–related protein (LRP) (Moestrup et al., 1990). Thus, α_2M may serve as a humoral or tissue binding or carrier protein for targeting peptides to their sites of action or clearing them from tissues.

Increased deposition of amyloid is one of the principle neuropathological features of Alzheimer's disease (AD) (Selkoe, 1994). Amyloid deposits are composed primarily of a 39–43-amino-acid peptide(s), the β-amyloid peptide (Aβ), which is a proteolytic processing product(s) of a larger integral transmembrane protein, the amyloid precursor protein (APP) (Kang et al., 1987). The Aβ peptide(s), once formed, can oligomerize and aggregate into insoluble fibrils that are directly toxic to neurons (Pike et al., 1991). Overproduction of Aβ after overexpression of a mutant APP transgene in mice results in amyloid deposition and neurodegeneration that is reminiscent of that seen in

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Abbreviations used: Aβ, β-amyloid peptide; AD, Alzheimer's disease; APP, amyloid precursor protein; BAPNA, sodium benzoyl-DL-arginine-*p*-nitroanilide; BSA, bovine serum albumin; IL, interleukin; LRP, low-density lipoprotein receptor–related protein; α_2M, α_2-macroglobulin; α_2M-MA, methylamine-activated α_2M; α_2M-T, trypsin-activated α_2M; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

AD (Games et al., 1995). However, overproduction of Aβ (due to either increased expression of APP or processing to Aβ) does not appear to occur in the vast majority of patients with AD, suggesting that differences in either the metabolism or clearance of Aβ, or both, may underlie the deposition of amyloid which invariably occurs in AD (Van Gool et al., 1995).

In addition to amyloid, other proteinaceous constituents of senile plaques have been identified. One of these, α_2M, is up-regulated in AD brain where it has been localized to neuritic but not diffuse (preamyloid) plaques (Strauss et al., 1992; Van Gool et al., 1993). Moreover, the α_2M receptor or LRP is also expressed in brain (in both neurons and glia) and is especially abundant in areas such as the hippocampus and entorhinal cortex, which are selectively affected in AD (Rebeck et al., 1993; Tooyama et al., 1993). In light of the well-described capacity of α_2M to bind various peptides and cytokines, as well as its postulated role in the pathogenesis of AD (Strauss, et al., 1992; Van Gool et al., 1993), we examined the binding of Aβ to α_2M.

MATERIALS AND METHODS

Materials

Synthesized human $A\beta_{(1-42)}$ was purchased from Bachem (Torrance, CA, U.S.A.). Human α_2M was purchased from Calbiochem (La Jolla, CA, U.S.A.). Methylamine, sodium benzoyl-DL-arginine-*p*-nitroanilide (BAPNA), α_1-antichymotrypsin, anti-α_2M antiserum, porcine chymotrypsin, and trypsin were from Sigma (St. Louis, MO, U.S.A.). Microcon microconcentrators were from Amicon (Beverly, MA, U.S.A.).

Iodination of $A\beta_{(1-42)}$

$A\beta_{(1-42)}$ was radiolabeled with $Na^{125}I$ according to the chloramine T–labeling method of Du Pont Medical Products (Boston, MA, U.S.A.) (sp. act. 2,200 Ci/mmol) (Banks et al., 1991). In brief, $A\beta_{(1-42)}$ peptide was incubated with $Na^{125}I$ and chloramine T in phosphate-buffered saline (PBS), pH 7.5, for 2 min. The reaction was quenched by the addition of sodium metabisulfite and purified by HPLC (99% purity), ^{125}I-$A\beta_{(1-42)}$ was stored in PBS/0.1% bovine serum albumin (BSA) at −80°C or −20°C for <2 months. The quality of ^{125}I-$A\beta_{(1-42)}$ was determined by native or sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

Activation of α_2M with methylamine or trypsin

Human α_2M was incubated at room temperature with 0.23 *M* methylamine for 110 min to yield methylamine-activated α_2M (α_2M-MA), or with a 4:1 molar excess of trypsin for 30 min to yield trypsin-activated α_2M (α_2M-T). All incubations were performed in a buffer consisting of 0.2 *M* HEPES (pH 8.0), 50 m*M* NaCl, and 2 m*M* EDTA. Excess methylamine, trypsin, or chymotrypsin was removed by membrane ultrafiltration (Bonner et al., 1992).

Binding of ^{125}I-$A\beta_{(1-42)}$ to α_2M

α_2M (10–400 μg/assay) was incubated with 0.2–1.0 ng ^{125}I-$A\beta_{(1-42)}$ in PBS containing 0.2% Triton X-100 at 37°C for 2 h in a total incubation volume of 40 μl for gel electrophoresis analysis or 200 μl for immunoprecipitation experiments.

Gel electrophoresis

Electrophoresis of the ^{125}I-$A\beta/\alpha_2$M complex in a native gel (5% Tris-borate) was performed as previously described (Nelles et al., 1980). Samples were electrophoresed using 10% PAGE for 2–3 h at 150–200 V, subsequently stained with Coomassie Brilliant Blue, and dried before autoradiography. To quantify the radioactivity of the complex, the stained bands of interest were removed with a razor blade and counted in a γ-scintillation counter.

Trypsin binding assay for α_2M

The purity of native α_2M was tested by the assay of Ganrot (1966). Both α_2M and α_2M-MA were tested for trypsin binding activity by a previously described method (Bonner et al., 1992). Increasing concentrations (50 μl/well) of α_2M in 25 m*M* Tris-HCl, 150 m*M* NaCl, pH 7.4, were incubated with 3 μl/well of 1 mg/ml trypsin for 10 min and then 6 μl/well of 1 mg/ml soybean trypsin inhibitor was added. After 10 min, 80 μl of 0.1 *M* Tris-HCl, 10 m*M* $CaCl_2$ buffer, pH 8.0, was added, followed by 100 μl/well 3 m*M* BAPNA. The reaction was stopped by the addition of 10 μl of glacial acetate and read at 405 nm.

Immunoprecipitation experiments

The ^{125}I-$A\beta/\alpha_2$M reaction mixture (200 μl) was incubated with 20 μl of anti-α_2M antiserum (or control serum) at 4°C overnight. Immunoprecipitates were centrifuged at 10,000 *g* and washed five times with binding buffer before scintillation counting.

Separation of ^{125}I-$A\beta_{(1-42)}$ and α_2M using HPLC

The binding of ^{125}I-$A\beta_{(1-42)}$ and purified α_2M or α_2M present in human serum was analyzed by HPLC gel-filtration chromatography using a modification of the method of Ikai et al. (1988). The equipment consisted of a TSK-Gel G4000PWXL column (7.8 mm i.d. × 30 mm; TosoHass, Montgomeryville, PA, U.S.A.) and a Beckman System Gold Chromatograph with in-line UV detection (Beckman Instruments, San Ramon, CA, U.S.A.). After incubation at 37°C for 2 h, the sample was filtered by microconcentration with a 300-kDa filter to remove free ^{125}I-$A\beta_{(1-42)}$. The recovered ^{125}I-$A\beta/\alpha_2$M complex was dissolved in PBS, then isocratically eluted in a mobile phase containing 150 m*M* NaCl and 50 m*M* NaH_2PO_4, pH 6.8. α_2M was quantified by absorbance at 280 nm. ^{125}I-$A\beta_{(1-42)}$ content was determined by measurement of radioactivity in sequentially collected fractions. To measure binding of ^{125}I-$A\beta_{(1-42)}$ to α_2M present in human serum, the fraction corresponding to α_2M was collected as described above and then filtered to remove albumin and other smaller proteins. The $A\beta/\alpha_2$M complex recovered was then rechromatographed in the same manner as for purified α_2M.

Effect of trypsin on the ^{125}I-$A\beta/\alpha_2$M-MA complex

α_2M-MA (40 μg) was incubated with ^{125}I-$A\beta_{(1-42)}$ for 2 h at 37°C in the same binding buffer as described above. The resulting sample was then treated with various concentrations of trypsin for 1 h at 37°C and the amount of bound ^{125}I-$A\beta_{(1-42)}$ analyzed by using a native 5% gel or by immunoprecipitation as described above. Control incubations were performed in an identical fashion in the absence of α_2M-MA or with control antiserum.

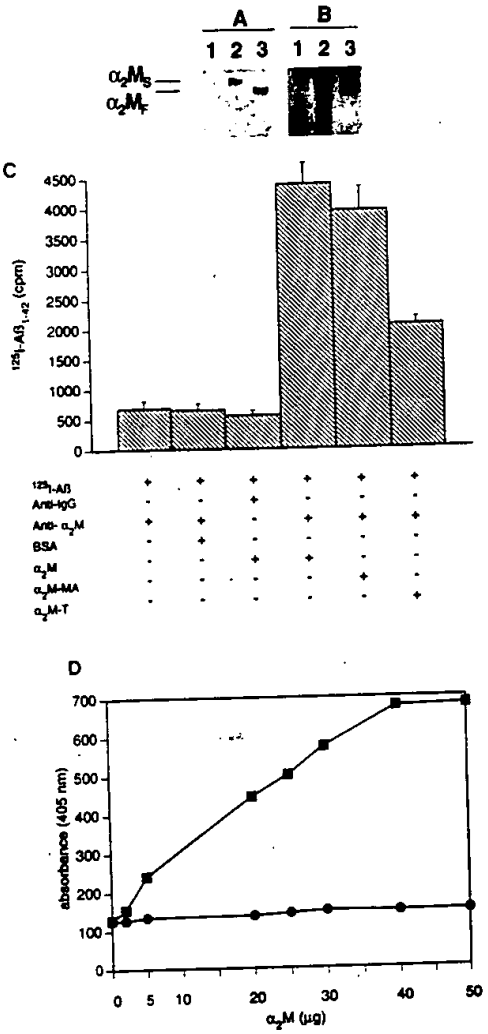

FIG. 1. The binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M. Human α_2M (1 mg/ml) was incubated with ^{125}I-A$\beta_{(1-42)}$ (5 ng/ml) and the bound peptide/α_2M measured as described under Materials and Methods. **A** (Coomassie Blue staining) and **B** (autoradiogram of a typical gel): Lane 1 corresponds to ^{125}I-A$\beta_{(1-42)}$ alone. Lanes 2 and 3 show that ^{125}I-A$\beta_{(1-42)}$ binds to native α_2M (slow form) and to α_2M-MA (fast form). **C:** Immunoprecipitation of ^{125}I-A$\beta_{(1-42)}$/α_2M complex with rabbit anti-α_2M antiserum. Human α_2M (1 mg/ml) was incubated with ^{125}I-A$\beta_{(1-42)}$ (5 ng/ml). The amount of immunoprecipitable ^{125}I-A$\beta_{(1-42)}$ was reduced by ≥60% when incubated with α_2M-T compared with native α_2M or α_2M-MA. Background precipitation was <15% of control. Each experiment was repeated three times with similar results. **D:** Trypsin binding assay for α_2M. Native α_2M (■) possesses trypsin binding activity, whereas α_2M-MA (●) does not bind trypsin.

RESULTS

Using native gel electrophoresis, we observed binding of ^{125}I-A$\beta_{(1-42)}$ to purified human α_2M. As shown in Fig. 1, under our incubation conditions, ^{125}I-A$\beta_{(1-42)}$ binds to native α_2M and α_2M-MA (Fig. 1B, lanes 2 and 3). To further characterize the binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M, rabbit anti-human α_2M polyclonal antiserum was used to immunoprecipitate the ^{125}I-A$\beta_{(1-42)}$/α_2M binding complex. From Fig. 1B it is clear that a significant amount of radioactivity can be immunoprecipitated by using an antiserum to human α_2M. However, only background radioactivity is precipitated with an antiserum to human IgG or when BSA is substituted for α_2M. Similar to the data using native gel electrophoresis to separate bound from free ^{125}I-A$\beta_{(1-42)}$, it appears that ^{125}I-A$\beta_{(1-42)}$ binds to native α_2M and α_2M-MA, whereas less binding to α_2M-T is observed (Fig. 1C). For convenience, we used α_2M-MA for most of the subsequent experiments. To confirm that native α_2M was of good quality and had been completely converted to activated α_2M after treatment with methylamine, the trypsin binding assay for α_2M was also used (Bonner et al., 1992). The data demonstrated that >90% of native α_2M in selected lots possessed good trypsin binding activity that is proportional to the increasing amount of slow α_2M, but methylamine treatment inhibited this binding capacity (Fig. 1D).

To characterize the specificity of Aβ binding to α_2M, competition experiments designed to examine the effects of various peptides and cytokines on the binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M-MA were performed. Binding was examined in the presence of a 10^3-fold molar excess of unlabeled A$\beta_{(1-42)}$ (Fig. 2), A$\beta_{(1-40)}$ or A$\beta_{(40-1)}$ (data not shown), or a ~3,000-fold molar excess of interleukin 1β (IL-1β) or interleukin 2 (IL-2) (data not shown). The binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M-MA is observed in the absence of unlabeled A$\beta_{(1-42)}$ but is completely inhibited by concentrations of A$\beta_{(1-42)}$ ≥ 1.1 μM (≥44 pmol/assay) (Fig. 2). Moreover, A$\beta_{(1-40)}$, not A$\beta_{(40-1)}$ can also effectively inhibit the binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M. Finally, little to no inhibition of ^{125}I-A$\beta_{(1-42)}$ binding to α_2M is observed with high concentrations of IL-1β or IL-2, two cytokines that have been previously shown to bind to α_2M (Borth and Luger, 1989; Legrés et al., 1995).

The binding of ^{125}I-A$\beta_{(1-42)}$ to various concentrations of α_2M was further investigated by using immunoprecipitation. The binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M increases with increasing concentrations of α_2M, such that saturation of binding [0.2 ng ^{125}I-A$\beta_{(1-42)}$ (0.05 pmol)] is reached at 200 μg α_2M (276 pmol) (Fig. 3). To determine the time required for ^{125}I-A$\beta_{(1-42)}$ binding to α_2M to reach equilibrium, binding was performed at pH 7.4 [0.2 ng ^{125}I-A$\beta_{(1-42)}$ (0.05 pmol) and 40 μg α_2M (55 pmol)] and the amount of binding was determined at various time points (0.5–24 h) by

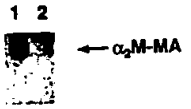

FIG. 2. Competition experiments. Competition studies were conducted at a constant ratio of ^{125}I-A$\beta_{(1-42)}$/α_2M-MA [5 ng/ml (1 nM)/1 mg/ml (1.4 μM)]. The binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M-MA was performed in the absence (1) or presence (2) of 5 μg/ml (1 μM) of unlabeled A$\beta_{(1-42)}$. These competition experiments were repeated twice with similar results.

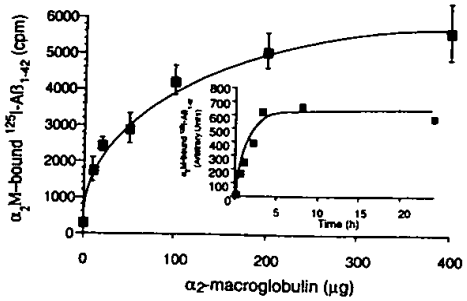

FIG. 3. Concentration- and time-dependent binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M. ^{125}I-A$\beta_{(1-42)}$ (5 ng/ml) was incubated with various amounts of α_2M at 37°C for 2 h. The complex was immunoprecipitated by incubation with anti-α_2M antiserum. The amount of radioactivity of the ^{125}I-A$\beta_{(1-42)}/\alpha_2$M complex was determined by scintillation counting. **Inset:** Time course of ^{125}I-A$\beta_{(1-42)}$ binding to α_2M. α_2M (1 mg/ml) was incubated with ^{125}I-A$\beta_{(1-42)}$ (5 ng/ml) for various times (0.5–24 h) at 37°C in 40 μl of the binding buffer, pH 7.4. Bound from free ^{125}I-A$\beta_{(1-42)}$ was separated by gel electrophoresis (see text for details). The amount of radioactivity was determined by direct scintillation counting of the excised bands.

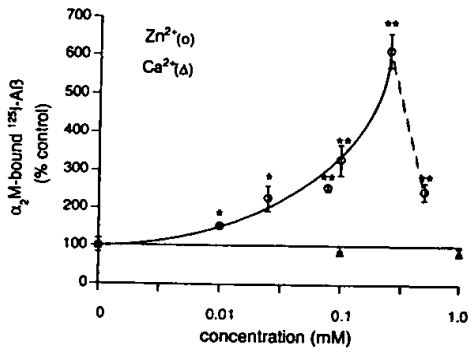

FIG. 5. Effects of Zn^{2+} and Ca^{2+} on the binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M-MA. ^{125}I-A$\beta_{(1-42)}$ (5 ng/ml) was incubated with α_2M-MA (1 mg/ml) in 100 m*M* Tris-HCl buffer (pH 8.0) at 37°C for 2 h in the presence of various concentrations of Ca^{2+} or Zn^{2+}. Bound ^{125}I-A$\beta_{(1-42)}$ was immunoprecipitated and the radioactivity determined as described in the text. Note that low concentrations of Zn^{2+} increase ^{125}I-A$\beta_{(1-42)}$ binding to α_2M-MA, whereas high concentrations directly precipitate ^{125}I-A$\beta_{(1-42)}$ or α_2M-MA, resulting in a less immunoprecipitable ^{125}I-A$\beta_{(1-42)}/\alpha_2$M-MA complex. $^*p < 0.05$; $^{**}p < 0.01$, by Student's *t* test.

using native gel electrophoresis as described above (see inset). The results indicate that ^{125}I-A$\beta_{(1-42)}$ binds to α_2M with a $t_{1/2}$ of ~90 min and equilibrium is reached at ~4 h. Using 40 μg α_2M (55 pmol)/assay, we varied the concentration of ^{125}I-A$\beta_{(1-42)}$ and separated bound from free radioligand by native gel electrophoresis. A saturation isotherm of "specific" binding is shown in Fig. 4. The apparent dissociation constant and maximal binding capacity for ^{125}I-A$\beta_{(1-42)}$ binding to α_2M were estimated by Scatchard analysis (Fig. 4, inset), yielding an apparent dissociation constant (K_D) of ~3.8×10^{-10} *M* and an apparent B_{max} of ~1.1 binding sites of Aβ/mol of α_2M.

To explore incubation conditions and cofactors that

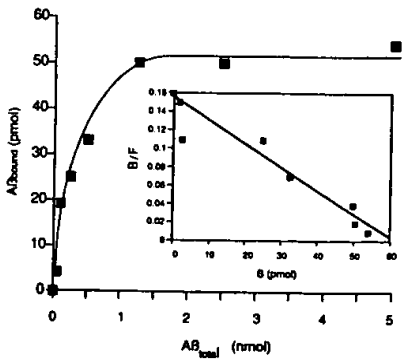

FIG. 4. Saturation isotherm and Scatchard analysis of ^{125}I-A$\beta_{(1-42)}$ binding to α_2M. ^{125}I-A$\beta_{(1-42)}$ (5 ng/ml) with or without cold A$\beta_{(1-42)}$ (0.1–5 n*M*) was incubated with native human α_2M (1 mg/ml) for 4 h as described in the text. Bound from free radioligand was determined by nondenaturing gel electrophoresis as described in the text. Scatchard analysis of the binding data (**inset**) yielded an apparent dissociation constant of 3.8×10^{-10} *M*. Data are from a representative experiment repeated twice with similar results.

may affect the binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M, we examined the effects of pH and the cations Ca^{2+} and Zn^{2+} on the binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M by using the immunoprecipitation method. The binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M-MA was found to be dependent on pH. The binding complex being relatively stable over the pH range examined (5.0–8.0, data not shown), however, the absolute amount of ^{125}I-A$\beta_{(1-42)}$ bound to α_2M-MA increases by ~60% as the pH is decreased from 7.5 to 5.0. This finding was confirmed by using native gel electrophoresis to separate bound from free ligand (data not shown).

Previous studies have shown that both α_2M and Aβ can bind Zn^{2+} (Parisi and Vallee, 1970; Bush et al., 1994), and we therefore examined whether the formation of the ^{125}I-A$\beta_{(1-42)}/\alpha_2$M complex is influenced by Zn^{2+}. In the presence of Zn^{2+} (0.01–0.25 m*M*) the binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M-MA is increased in a concentration-dependent fashion (Fig. 5). At low [Zn^{2+}] (i.e., ≤0.25 m*M*), we observed enhanced binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M, whereas higher Zn^{2+} concentrations (>0.25 m*M*) resulted in a direct precipitation of the ^{125}I-A$\beta_{(1-42)}$, yielding lower amounts of immunoprecipitable complex (Fig. 5). By contrast, Ca^{2+} concentrations of ≤1 m*M* had no effect on the binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M (Fig. 5).

Because the binding of IL-2 to α_2M-MA has been shown to protect this peptide substrate from exogenous proteinases (Legrés et al., 1995), we subjected the ^{125}I-A$\beta_{(1-42)}/\alpha_2$M-MA complex to treatment with various concentrations of trypsin. Both gel and immunoprecipitation studies revealed that ^{125}I-A$\beta_{(1-42)}$ resisted trypsin digestion when bound to α_2M-MA (Fig. 6). By contrast, unbound ^{125}I-A$\beta_{(1-42)}$ was readily degraded by trypsin.

We have also examined whether the plaque-associ-

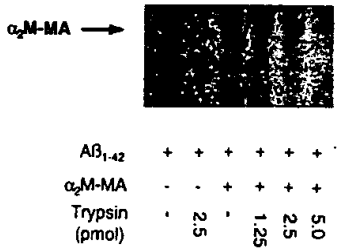

FIG. 6. Effect of trypsin on ^{125}I-A$\beta_{(1-42)}$ bound to α_2M-MA. Preincubation of ^{125}I-A$\beta_{(1-42)}$ (5 ng/ml) with α_2M-MA (1 mg/ml) protects the peptide from subsequent proteolysis by trypsin. The autoradiogram shows that complexes of ^{125}I-A$\beta_{(1-42)}$ with α_2M or α_2M-MA resist digestion by 1.25, 2.50, and 5.0 pmol of trypsin.

ated protein α_1-antichymotrypsin affects the formation of the ^{125}I-A$\beta_{(1-42)}/\alpha_2$M complex. In these experiments, various amounts of α_1-antichymotrypsin were added to the binding reaction before equilibrium. Unexpectedly, with increasing concentrations of α_1-antichymotrypsin, the amount of ^{125}I-A$\beta_{(1-42)}/\alpha_2$M complex decreased dramatically (Fig. 7), suggesting that α_1-antichymotrypsin is able to inhibit the binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M.

Finally, to determine whether A$\beta_{(1-42)}$ can bind to α_2M present in serum, we incubated human serum with ^{125}I-A$\beta_{(1-42)}$ (2 h, 37°C) and attempted to immunoprecipitate or separate the ^{125}I-A$\beta_{(1-42)}/\alpha_2$M complex by using anti-α_2M antiserum or HPLC. From Fig. 8, it is apparent that after incubation of human serum with ^{125}I-A$\beta_{(1-42)}$, immunoprecipitation of α_2M with anti-α_2M antiserum (A) or separation of α_2M by using HPLC (B) reveals binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M present in serum. These data demonstrate that in the presence of other serum proteins ^{125}I-A$\beta_{(1-42)}$ can bind to α_2M.

DISCUSSION

Aβ, the main component of neuritic plaques, which are characteristic features of AD neuropathology, is also present as a normal constituent of biological fluids and is apparently produced by many cells during normal cellular metabolism (Selkoe, 1994). The concentration of Aβ in biological fluids such as CSF is $\sim 10^{-9}$ M (Haass et al., 1992). Given that the circulating or brain concentrations of Aβ are different in the patients who subsequently develop AD, factors that determine or affect the catabolism or clearance of Aβ (or both) may contribute to Aβ aggregation and deposition into either diffuse (preamyloid) and (or) neuritic (senile) plaques in brain (Van Gool et al., 1993; Selkoe, 1994; Tabaton et al., 1994; Kuo et al., 1996). In the present study, we have found that the major circulating panproteinase inhibitor α_2M is capable of binding ^{125}I-A$\beta_{(1-42)}$ with an apparent dissociation constant of $\sim 3.8 \times 10^{-10}$ M and, therefore, may bind or sequester Aβ at the low concentrations of this peptide(s) that occur physiologically. Moreover, the binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M appears to be relatively specific in that binding can be inhibited by coincubation with na-

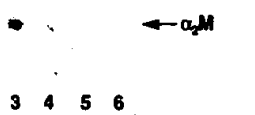

FIG. 7. Effects of α_1-antichymotrypsin on the binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M. In lanes 1 and 2, 0.2 ng ^{125}I-A$\beta_{(1-42)}$ was incubated with 0 and 20 μg α_1-antichymotrypsin only. In lanes 3, 4, 5, and 6, which represent binding reactions (40 μl) with 0, 5, 10, and 20 μg α_1-antichymotrypsin, respectively, α_1-antichymotrypsin was added to binding reactions consisting of 0.2 ng ^{125}I-A$\beta_{(1-42)}$ and 40 μg α_2M. With increasing amounts of α_1-antichymotrypsin (0, 5, 10, and 20 μg), the binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M decreased, as revealed by the autoradiogram.

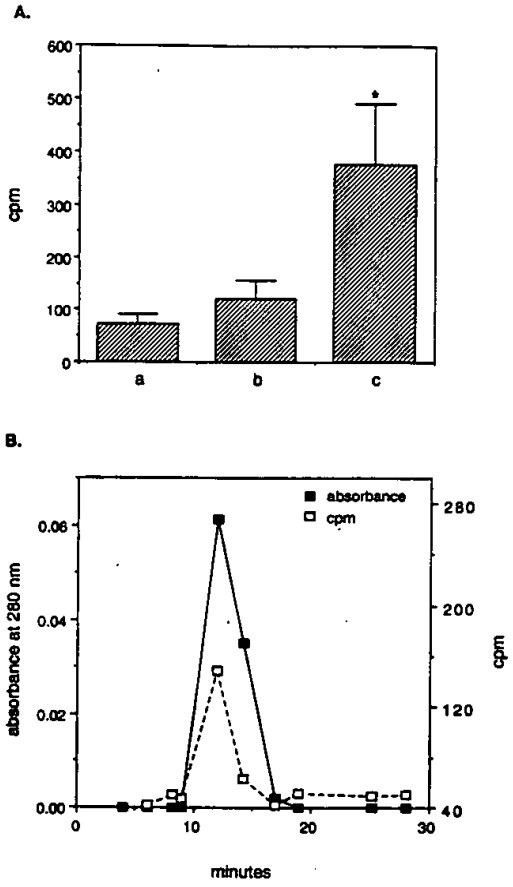

FIG. 8. Binding of ^{125}I-A$\beta_{(1-42)}$ to α_2M present in human serum. **A:** ^{125}I-A$\beta_{(1-42)}$ (50 ng/ml) was incubated with human serum (100 μl) at 37°C for 2 h. Immunoprecipitation of the ^{125}I-A$\beta_{(1-42)}/\alpha_2$M complex from human serum with anti-α_2M antiserum. Column a, ^{125}I-A$\beta_{(1-42)}$ and human serum; column b, ^{125}I-A$\beta_{(1-42)}$, human serum, and anti-human IgG antibody; and column c, ^{125}I-A$\beta_{(1-42)}$, human serum, and anti-human α_2M antiserum. $p < 0.05$, by Student's t test. **B:** ^{125}I-A$\beta_{(1-42)}$ (50 ng/ml) was incubated with human serum (100 μl) at 37°C for 2 h. The sample was analyzed by HPLC as described in Materials and Methods. The resulting α_2M-containing fractions were associated with ^{125}I-A$\beta_{(1-42)}$.

nomolar concentrations of $A\beta_{(1-42)}$ or $A\beta_{(1-40)}$ but not by the reverse peptide $A\beta_{(40-1)}$ or by cytokines IL-1β and IL-2. Both IL-1β and IL-2 have been previously shown to bind with relatively high affinity to α_2M (Borth and Luger, 1989; Legrés et al., 1995). These data also confirm that the noniodinated $A\beta_{(1-40)}$ or $A\beta_{(1-42)}$ peptides can effectively compete with ^{125}I-$A\beta_{(1-42)}$ in binding to α_2M and, therefore, the ability of Aβ to bind to α_2M does not appear to be due to peptide iodination.

The binding of ^{125}I-$A\beta_{(1-42)}$ to α_2M does not appear to require proteolytic activation of α_2M, because both native α_2M and α_2M-MA bind ^{125}I-$A\beta_{(1-42)}$ (Fig. 1C). These findings are reminiscent of those recently reported by Legrés and colleagues (1995) who demonstrated similar high affinity binding of ^{125}I-recombinant human IL-2 to native and α_2M-MA. By contrast, only activated α_2M is capable of binding to IL-1β (Borth and Luger, 1989).

Because both α_2M and Aβ have been reported to bind Zn^{2+}, we examined the effects of Zn^{2+} on ^{125}I-$A\beta_{(1-42)}$ binding to α_2M. Relatively low concentrations of Zn^{2+} (0.01–0.25 mM) enhance the binding of ^{125}I-$A\beta_{(1-42)}$ to α_2M, whereas higher concentrations (>0.25 mM) directly precipitate the ^{125}I-$A\beta_{(1-42)}$ from solution (data not shown). By contrast, $[Ca^{2+}]$ of $\leq$1 mM fails to alter the binding of ^{125}I-$A\beta_{(1-42)}$ to α_2M (Fig. 6). Although the postulated role of Zn^{2+} in the pathogenesis of AD is controversial (Lui et al., 1990), our data suggest that low physiologically relevant $[Zn^{2+}]$ may facilitate the formation of the Aβ/α_2M complex. The latter may facilitate Aβ deposition into plaques containing α_2M (see below).

It is interesting that similar to the cytokine IL-2, the binding of ^{125}I-$A\beta_{(1-42)}$ to α_2M protects the peptide from proteolysis by exogenous trypsin (Fig. 6). It was postulated that the trypsin-sensitive sites on the peptide became inaccessible after binding to α_2M-MA. We have found that α_1-antichymotrypsin (another plaque-associated protein) (Abraham et al., 1988) also blocks the proteolysis of Aβ by α_2M-T (Zhang et al., 1996). Curiously, in addition to inhibiting α_2M-T–induced catabolism of Aβ, α_1-antichymotrypsin inhibits the binding of ^{125}I-$A\beta_{(1-42)}$ to α_2M (Fig. 7). It is possible that the previously reported properties of α_1-antichymotrypsin to bind to Aβ may reduce the binding of ^{125}I-$A\beta_{(1-42)}$ to α_2M (Fraser et al., 1993). Finally, ^{125}I-$A\beta_{(1-42)}$ binding to α_2M can also be demonstrated after incubation of ^{125}I-$A\beta_{(1-42)}$ with human serum in vitro, and thus, the binding of ^{125}I-$A\beta_{(1-42)}$ to α_2M occurs in the presence of other plasma proteins (Fig. 8). Although it is possible (perhaps likely) that other plasma proteins will bind Aβ, these experiments, in our view, demonstrate the Aβ binding capacity of α_2M, even in the presence of other potential competing binding proteins or catabolic enzymes. In addition, we have not addressed the possibility that iodination of Aβ may interfere with the conformation of this peptide and its binding ability (Bush et al., 1994). Further studies using more conservative labeling methods are under way.

What, if any, is the physiological or pathophysiological significance of the binding of Aβ to α_2M? How could such binding be relevant (if at all) to the pathogenesis of AD? At present, we can only speculate as to the possible significance of Aβ binding to α_2M. Because Aβ is normally produced by many cell types and because native α_2M can bind (with high affinity) the relatively low concentrations of circulating or tissue Aβ, it is likely that under normal (physiological) conditions native α_2M can sequester or bind Aβ. Conceivably, after activation of α_2M by proteinases, the associated (and protected) Aβ would be "cleared" via the Ca^{2+}-dependent binding and endocytosis of the Aβ/α_2M complex, i.e., by the α_2M/LRP. Thus, the binding of Aβ/α_2M to the LRP could serve to remove or clear Aβ from tissues, especially the brain, which expresses relatively high amounts of LRP (Tooyama et al., 1993). Alternatively, such binding and subsequent LRP-mediated endocytosis of the Aβ/α_2M complex by neurons (as opposed to macrophages) could result in enhanced deposition, especially if Aβ catabolism or binding to α_2M is inhibited by endogenous inhibitors such as α_1-antichymotrypsin. It is significant that α_1-antichymotrypsin has been reported to be present in amyloid-containing plaques and is expressed by astrocytes in affected (but not unaffected) areas of AD brain (Das and Potter, 1995). Thus, α_2M may serve to protect Aβ from proteolytic degradation and contribute to its deposition in plaques. Finally, it is also possible that the clearance of the Aβ/α_2M complex is affected by other LRP ligands, especially apolipoprotein E (Van Gool et al., 1995). An isoform-specific competition by apolipoprotein E–containing chylomicrons could further facilitate Aβ deposition and contribute to the pathogenesis of AD. The latter may contribute to the earlier onset of AD in individuals with either one or two copies of the apolipoprotein E4 allele (Strittmatter and Roses, 1995). These hypotheses are currently being investigated.

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